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OBSERVATIONS

ON THE

# STRUCTURE OF NERVE-FIBRES.

BY

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AND

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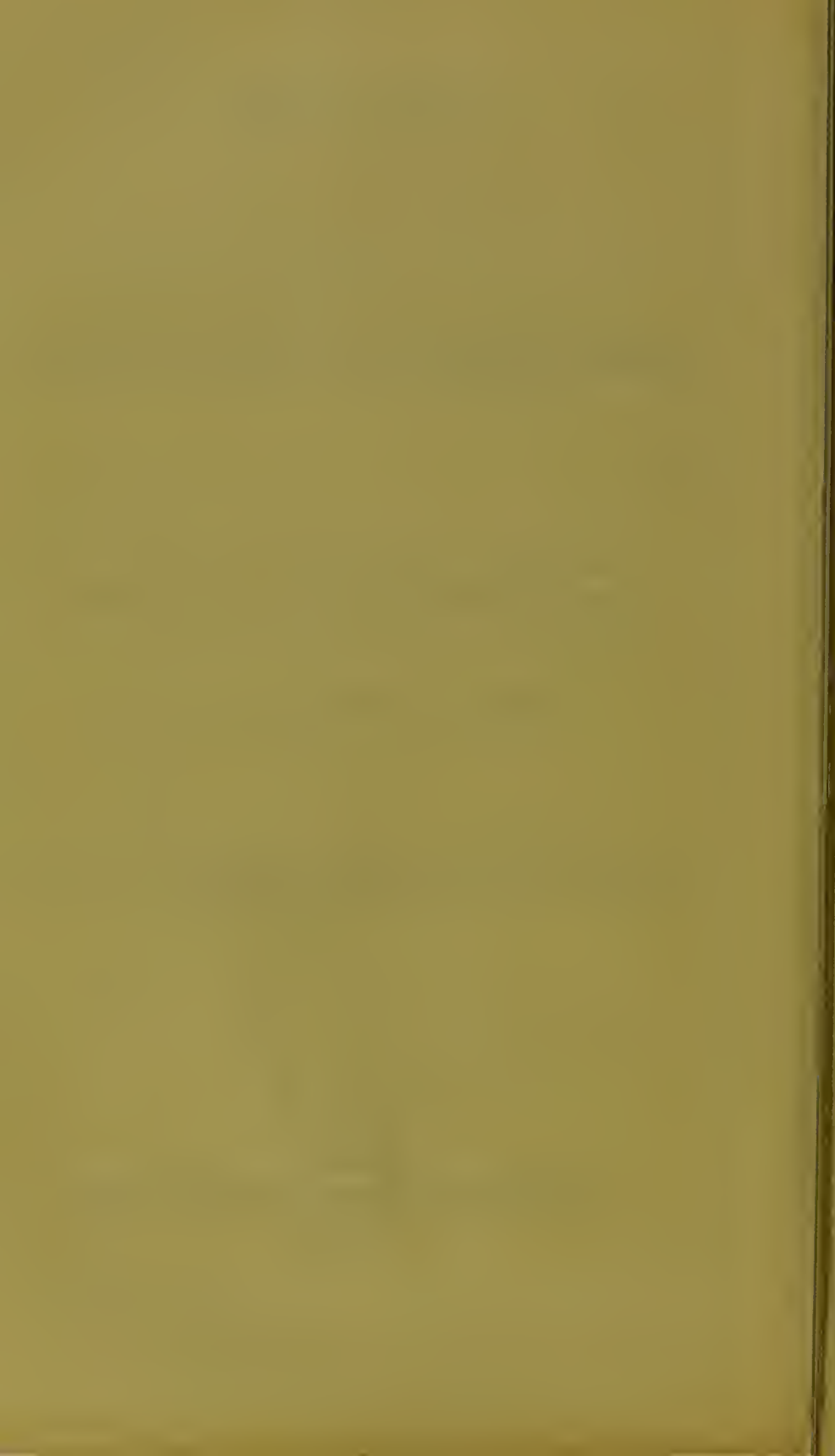
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*Some Observations on the STRUCTURE of NERVE-FIBRES.*

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HAVING recently had the opportunity, through the kindness of Mr. Loekhart Clarke, of inspecting some of his beautiful preparations of the spinal cord, we were struck with an appearance which had not yet received a satisfactory interpretation; and, having been induced to investigate the point, we have met with some facts which seem of sufficient interest for publication.

For the sake of clearness it may be well to state briefly the method employed by Mr. Clarke in preparing his specimens.

A portion of perfectly fresh spinal cord having been hardened by steeping in dilute chromic-acid solution, thin sections are made with a razor, and these, after immersion for a while in an ammoniacal solution of carmine, are soaked in spirits of wine to remove the water, and then treated with oil of turpentine. The last-named agent has the effect of rendering the sections transparent, so that the nerve-cells of the gray matter, finely coloured by the carmine, are seen with the utmost distinctness, giving off in various directions long branching processes; while the nerve-fibres, which are similarly tinted, may be traced with equal facility in their course through the cord.

In the preparations which we saw, the cord had been sliced crosswise, and in the columnar regions, where the nerve-fibres have for the most part a longitudinal direction, the transverse section of each fibre showed itself as a carmine-coloured point, surrounded by a perfectly pellucid and colourless ring. This was the appearance which seemed to demand explanation; the question being whether the transparent ring was a mere space, resulting from shrinking of the object during the preparation, or the white substance of Schwann (medullary sheath) rendered transparent by the turpentine, the axial cylinder alone, in that case, having received the carmine colour.

It occurred to us that the point might probably be determined by applying a similar mode of preparation to some nerve the dimensions of whose fibres could be readily ascer-

tained. With this view we steeped in chromic acid portions of the sciatic nerve of a cat just killed, and also parts of the spinal cord of the same animal; and having allowed them to remain between three and four weeks in the solution, we commenced the investigation in July of the present year, 1859.

A transverse section of the hardened sciatic nerve having been placed for a time in the carmine solution and then dried, we submitted it, without the application of turpentine, to microscopic examination with a power of 130 diameters. Viewed by transmitted light, it appeared as a confused opaque mass; but, by reflected light, it exhibited the structure depicted in Pl. II, fig. 1,\* each nerve-fibre presenting in its section a carmine spot, surrounded by a yellowish-white, somewhat granular ring, which, though doubtless corresponding to the pellucid rings in the preparations of the cord before alluded to, was clearly composed of some solid material, in short of the white substance of Schwann altered by the action of the chromic acid.

We next examined sections of the cord treated in the same way, but found that these dry specimens were so incrustated with carmine that they gave no definite results. It happened, however, that one of the sections treated with carmine still remained moist, and, after washing away all superfluous colouring matter, we examined it by transmitted light. A very beautiful appearance now presented itself; carmine points being seen in the columnar regions, as in Mr. Clarke's preparations, surrounded by rings; but the latter, instead of being transparent like mere spaces, were dead white; the carmine points, on the other hand, appearing in the thinnest parts of the section as illuminated spots amid the general opacity. This is represented in fig. 5.

It will be seen from this sketch, which is drawn on the same scale as fig. 1, that the nerve-fibres varied very much in their diameter, the largest being of about the same size as those of the sciatic nerve, while others were of extreme minuteness; but in all cases in which they were sufficiently large to be distinguished, they had the same character of a white circle with a central carmine spot from one fourth to one third the diameter of the whole fibre. It was obvious that, in the cord, as in the sciatic nerve, the carmine central part of each fibre was the axial cylinder, and the opaque circumferential portion the medullary sheath; and, therefore,

\* This sketch, like the others illustrating this paper, was drawn by means of the camera lucida.

that the pellucid rings in preparations treated with turpentine consisted of the white substance rendered transparent by that reagent.

The point at issue was thus satisfactorily decided ; but for the sake of confirmation we made some further observations, the results of which seem deserving of mention.

On examining the hardened sciatic nerve, without tinting the preparations with carmine, we found that in extremely thin slices the transverse sections of the nerve-fibres, viewed by transmitted light, appeared as brownish rings with central transparent colourless spots (see fig. 3), whilst by reflected light the central parts appeared black, as shown in fig. 2. In fact, under a low power the axial cylinders had, in these specimens of the sciatic nerve, as much the appearance of mere spaces as the medullary sheaths had in preparations of the cord treated with turpentine. But on applying a fine glass of high power, a granular appearance was disclosed in the pellucid central portion, showing that it was in reality a solid substance, though of a transparency which was very remarkable, considering that it had been so long subjected to the action of chromic acid ; and on afterwards treating similar sections with carmine we found that this part alone became coloured. The higher magnifying power also brought out an appearance of irregular concentric lines in the brown\* medullary sheath ; and this, together with the granular aspect of the axial cylinder, is represented in fig. 4.

These facts afford a very striking illustration of the essential difference in chemical composition between the axial cylinder and the medullary sheath ; the former being totally unaffected by chromic acid, though the latter is rendered opaque and brown and concentrically striated under its influence, while, on the other hand, the axial cylinder, after being subjected to the action of chromic acid, imbibes the carmine colour with peculiar facility, although the medullary sheath is entirely untinted by it.†

We next applied the high magnifying power to extremely thin slices of the spinal cord prepared in the same way. In transverse sections of the columnar regions the white

\* It must be mentioned that a similar brown colour is seen in the superficial parts of a cord which has been steeped in chromic acid, but the deeper portions of the organ are comparatively only slightly coloured, so that in individual nerve-fibres seen under a high magnifying power the brown tint is not observed.

† In a boiled fresh nerve also the medullary sheath remains unaffected by ammoniacal solution of carmine, while the axial cylinder assumes a distinct though very faint pink tint.—J. L.

substance of Schwann presented, in the larger fibres, the same concentrically arranged appearance as we had observed in the sciatic nerve, as is illustrated by figs. 6 and 7, of which fig. 6 is one of the largest met with, being  $\frac{1}{9000}$  of an inch in diameter, while fig. 7 is as small as  $\frac{1}{30000}$  of an inch in transverse measurement. In the very minute fibres no appearance of concentric lines could be detected, yet, wherever the existence of an axial cylinder was indicated by a carmine point, a ring of medullary sheath was always visible, presenting the same proportion to the axial cylinder as in fibres of larger size. This may be gathered from figs. 8, 9, and 10, of which fig. 8 measures  $\frac{1}{30000}$  of an inch across, fig. 9  $\frac{1}{80000}$ , and fig. 10 only  $\frac{1}{140000}$ .

At the margins of longitudinal sections of the cord, the contrast, both in structure and in tint, between the axial cylinder and the medullary sheath showed itself very beautifully. It often happened that a projecting isolated fibre was, near its extremity, more or less divested of the white substance of Schwann, so that the delicate, carmine-tinted axial cylinder was exposed, though presenting here and there colourless flakes of the medullary sheath adhering to its surface; while in parts where the nerve was still entire, the pink colour of the central fibre could be distinctly discerned through the intervening white substance. Fig. 11 represents a large fibre under such circumstances, and fig. 12 one of considerably smaller size; and these sketches also display the remarkable fibroid arrangement which we find the white substance of Schwann invariably assumes under the influence of chromic acid.

In conclusion, we may remark that the successive employment of chromic acid and carmine seems likely to afford valuable aid in discriminating nerve-fibres among other structures; there being, so far as we are aware, no other form of tissue which, after the use of these means, exhibits fibres having a central carmine axis, and peripheral uncoloured sheath.

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#### *Supplementary Observations by Mr. LISTER.*

The fibroid arrangement of the white substance of Schwann in nerves hardened by chromic acid has been minutely described by Stilling, in his elaborate treatise on the 'Nerve-fibre and Nerve-cell,'\* a work which we had not seen when

\* 'Ueber den Bau der Nerven-Primitivfaser und der Nervenzelle.' Von Dr. B. Stilling. 1856.

the foregoing communication was written, but a copy of which was kindly lent me by Professor Goodsir, soon after Mr. Turner had left Edinburgh for the vacation. According to Stilling, the medullary sheath is, even in perfectly fresh nerves, composed of a network of fibres, which are continuous with others in the axial cylinder and in the proper investing membrane; so that, in his opinion, these three constituents of the nerve-fibre differ from each other only in the manner in which their elements are disposed.\* This view is not only quite novel anatomically, but is opposed to the generally received physiological opinion, that the axial cylinder is the essential part of the nerve-fibre, and the medullary sheath an insulating investment. Considering the high estimation in which the writings of Stilling on the anatomy of the nervous centres are deservedly held, and the influence which therefore attaches to his opinions, it seems fortunate that we have been able to present so clear a demonstration that the axial cylinder is chemically as well as morphologically totally distinct from the medullary sheath.

With regard to the cause of the fibroid arrangement of the medullary sheath, an observation which I happened to make several years ago, regarding the aggregation of fatty matter, may perhaps tend to throw light upon the subject. I submitted to microscopic examination some of the pulsataceous slough of a sore affected with hospital gangrene, thinking it possible that I might discover in it some fungus which might account for the peculiar specific character of that disease; and found in it numerous bodies, each composed of branching fibres radiating from a common centre, and looking, at first sight, like some sort of vegetable growth, so that I made careful sketches of them, one of which is reproduced in fig. 13. But seeing afterwards, in the same object, some bundles of acicular crystals of margarine, having a distant resemblance to the bodies I had drawn, I added ether to the specimen, and found that it dissolved the latter equally with the former. This showed that what first attracted my attention was merely an arborescent form of aggregation of some fat, probably margarine; and it seems not unlikely that the fluid fat which exists in the medullary sheath of a perfectly fresh nerve, may tend to a similar arrangement of its particles when passing into the solid form, and so give rise to the appearance in question. It is to be remarked that the fibroid character is not peculiar to specimens treated with chromic acid, but also shows itself, though in a less perfect manner, in nerves which have been subjected to other modes

\* Op. cit., p. 6.

of preparation—for example, after exposure for a few seconds to a temperature of 212° F.

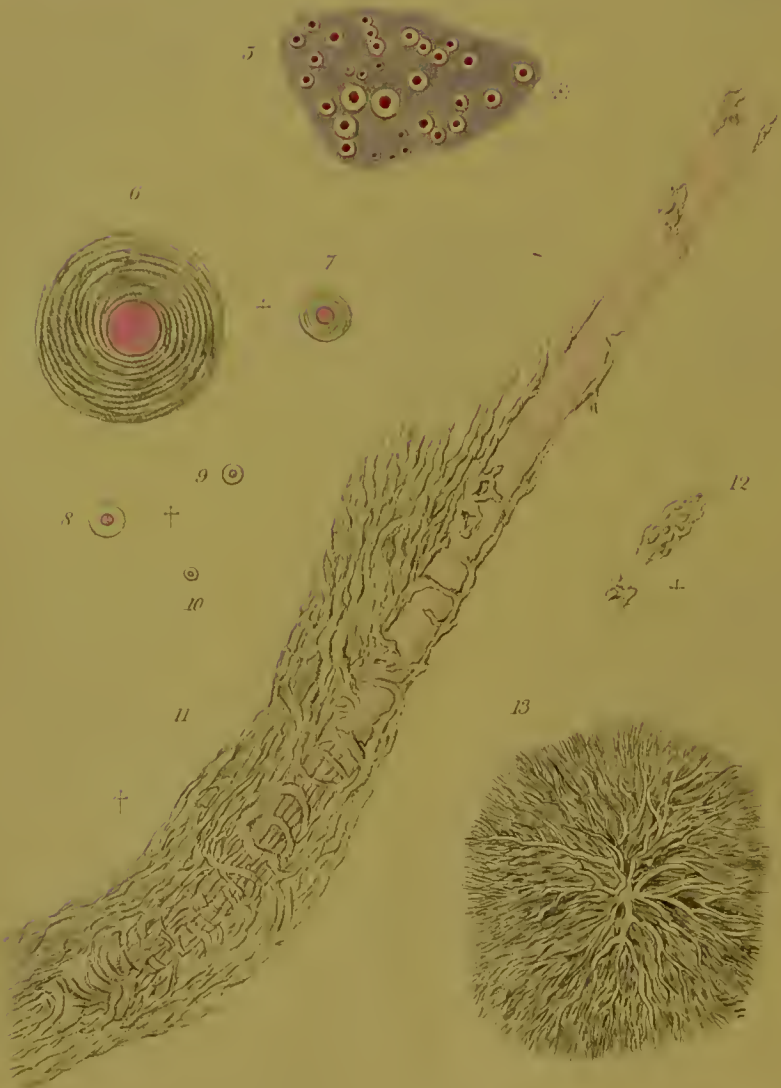
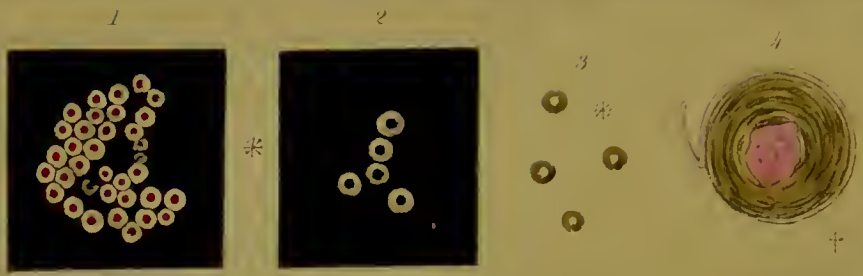
There is another important statement made by Stilling, which the use of the method of examination above described enables me to correct. He speaks of the fibres which connect one nerve-fibre with another as similar in every respect to those seen in the medullary sheath.\* I find, however, that both in the sciatic nerve and in the spinal cord of the cat, the connective tissue between the nerve-fibres, like the neurilemma and pia mater, with which it is continuous, becomes coloured by the carmine; whereas, the medullary sheath, as before stated, is quite unaffected by it, proving that the two structures are chemically distinct from one another. In both these situations, too, the fibres of the connective tissue are much more delicate than the constituents of the medullary sheath, which are often comparatively coarse, as may be seen from fig. 11. In the columnar regions of the cord, the former require a high magnifying power to be applied to very thin sections, in order to distinguish them, and are often present in such extremely small quantity that, without very careful examination, the nerve-fibres appear actually in contact with one another. In the sciatic nerve I have observed occasional elongated nuclei in the connective tissue.

I may add that glycerine has proved very useful, not only for permanently preserving the preparations in the moist state, but also as an aid to investigation; for it renders the sections much more transparent, without making the white substance of Schwann invisible, as turpentine does; and hence the course of the nerve-fibres through the cord can be traced much more easily, and, at the same time, the proportion between the medullary sheath and axial cylinder can be readily ascertained. Thus, by examining transverse sections of the cord in this way, I find that while Kölliker is quite correct in his statement that the fibres of the roots of the nerves diminish in size in passing inwards through the columnar regions,† yet the diminution affects only the white substance; the axial cylinder often retaining its full dimensions even in the middle of the gray matter, while the medullary sheath is reduced to a very thin crust, so that the nerve-fibre assumes a character differing but little from that of an offset of a nerve-cell.

\* Op. cit., p. 7.

† Kölliker's 'Handbuch der Gewebelehre,' 3d edit., p. 285.





⊗ 1/2 diameters  
 † 7/8

# JOURNAL OF MICROSCOPICAL SCIENCE.

## DESCRIPTION OF PLATE II,

Illustrating Messrs. Lister and Turner's paper on the  
Structure of Nerve-fibres.

Fig.

- 1.—Represents part of a transverse section of the sciatic nerve of a cat, hardened by chromic acid, and tinted with carmine; the axial cylinder alone having received the colouring matter. The specimen was dried and viewed as an opaque object.
- 2.—Shows the appearance of thin transverse sections of some nerve-fibres from the same nerve, simply hardened in chromic acid, and examined moist by reflected light. The axial cylinder has, under this low magnifying power, the aspect of a mere space.
- 3.—Similar objects to those of fig. 2, but seen by transmitted light.
- 4.—A highly magnified transverse section of a nerve-fibre from the same source, prepared like those of figs. 2 and 3, and then tinted with carmine. The carmine colour is seen to affect only the axial cylinder and the investing membrane, which, at one part, is torn up from the fibre. This sketch also shows the faintly granular structure of the axial cylinder, and the irregularly concentric striation of the medullary sheath.
- 5.—A transverse section of a columnar portion of the spinal cord of a cat, also prepared with chromic acid and carmine, and examined moist by transmitted light. The fibres vary much in size, but all of them resemble those of the sciatic nerve in having the red axial cylinder surrounded by a ring of untinted medullary sheath.
- 6—10 are highly magnified views of some fibres in a section of the cord like that of fig. 5. They present the same characters as the fibres of the sciatic nerve.
- 11.—A fibre from a longitudinal section of a columnar portion of the cord, prepared in the same way. The axial cylinder alone is carmine coloured, and is, in some parts, stripped of its investing sheath, the fibroid arrangement of which is also displayed.
- 12.—A small fibre under similar circumstances.
- 13.—Fatty matter in a state of arborescent fibroid aggregation.

